SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY SCREENING OF NOVEL SUBSTITUTED BENZIMIDAZOLES

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ABSTRACT : A series of novel benzimidazole (BM1-BM5) was designed, synthesized and characterized for evaluation of potential antibacterial activity against Bacillus substilis,Staphylococcus auras, (gram +ve) and Pseudomonas aeroginosa, Proteus vulgaris(gram –ve), Streptomycinused as standard drug and antifungal activity against Aspergillus niger and Trichoderma ressi at 100 μg/ml level, griseofulvin used as standard drug. Compound BM1 showed moderate activity against S. aureus and exhibited more activity against all bacteria. Compound BM5 showed as inactive against gram positive bacteria. Compounds BM1 and BM3 displayed better antifungal activity against 100 μg/ml concentration, all the test compounds showed nill antifungal activity at 50 μg/ml concentration. Therefore, compound BM1 can serve as a lead molecule for further development as new class of antimicrobial agent.
KEYWORDS:Benzimidazole, antibacterial & antifungal activity, Streptomycin and griseofulvin

I.INTRODUCTION

Heterocyclic ring system of benzimidazole and their derivatives aroused great interest for the past and recent years due to wide variety of biological properties such as antioxidant,[60] antimicrobial,[61] anthelmintic,[62] anticancer,[63] antihypertensive,[64] antineoplastic, antiinflammatory,[65] analgesic,[66] antiprotozoal,[67] anti-hepatitis B virus,[68] antiulcer,[69] antiviral,[70] antifungal,[71] anticonvulsant,[72] antivirus[73] and antihistaminics[74] activities. In view of these, we have planned to synthesize substituted benzimidazole by condensation of different 2-phenylenediamines and substituted aldehyde in presence of Zirconyl nitrate as a heterogeneous Lewis acid catalyst in ethanol. The synthesized derivatives were screened for antibacterial activity and antifungal activity with well-known organisms. Maximum benzimidazole derivatives showed better antibacterial activity and few of derivatives showed poor activity against positive and negative bacteria. In other side some of derivatives displayed better antifungal activity against 100 μg/ml concentration. The title compounds were synthesized by the following synthetic route depicted in scheme as given in experimental part.

II.MATERIAL & METHODS

Melting points (mp) were determined in open capillary tubes on Thomas Hoover melting point apparatus and are uncorrected. The IR spectra were recorded in potassium bromide disks using a Perkin-Elmer 398 spectrometer. The 1H NMR spectra were recorded on DPX-500 MHz Bruker FT-NMR spectrometer. The chemical shifts were reported in parts per million (δ ppm) relative to TMS as an internal reference. Mass spectra were recorded on a JEOLSX-102 instrument using fast atom bombardment (FAB positive). The progress of all the reactions were monitored by readymade silica gel plates (Merck) and a solvent system of petroleum ether/ethyl acetate (7:3). The spots were developed in iodine chamber. Spectral data (IR, NMR and Mass spectra) was confirmed the structures of the synthesized compounds and the purity of these compounds were ascertained by microanalysis. Elemental (C, H and N) analysis also indicated that the calculated and observed values were within the acceptable limits. All chemicals and reagents were procured from Aldrich (USA), Lancaster (UK) or SpectrochemPvt.Ltd (India) and were used without further purification.

III.EXPERIMENTAL

A general procedure for synthesis of substituted benzimidazoles:

0.5% weight of Zirconyl nitrate was added to a reaction mixture of aldehydes (1mmol) and 2-phenylenediamine

(1mmol) in ethanol (10ml). The reaction mixture was stirred under reflux for 2-6 h. The progress of the reaction was monitored by thin layer chromatography, using petroleum ether/ethyl acetate (7:3) as a solvent system. After completion of reaction, the reaction mixture was cooled to room temperature, 20 mL of ethanol was added and resulting solution was filtered to remove the catalyst. The filtrate was concentrated under reduced pressure, to separate the product and crystallized from ethanol. The structures of the benzimidazole were confirmed by FTIR, 1H NMR, 13C NMR, and HRMS and were mostly known compounds.

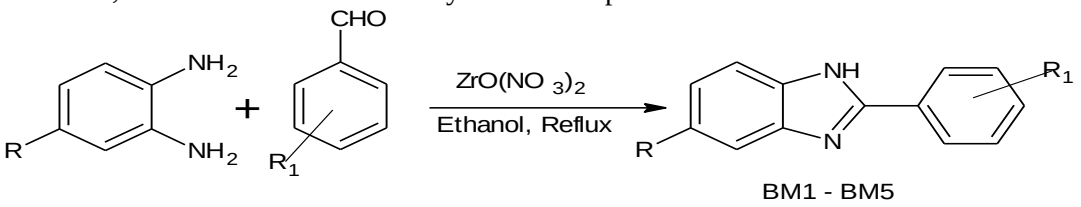

Figure: Synthetic scheme for synthesis of benzimidazole derivatives

Anti-microbial Activity

Anti-bacterial assay

For substituted-(tetrazolo[1,5-*a*] quinolin-4yl) derivatives **[BM1 to BM5]** the anti-bacterial activity was studied against the growth of *Bacillus substilis,Staphylococcus auras,* (gram +ve) and *Pseudomonas aeroginosa, Proteus vulgaris*(gram –ve) bacteria by the disc-fusion method[16] in nutrient agar medium at 100ppmconcentration in dimethyl sulfoxide (DMSO) **(Table 2)**. The results were comparedwith the activity of the standard anti-biotic Streptomycin (100mg/disc). Thesesolutions were added to each filter disc and DMSO was used as control for the bothseries of experiments.

The cultures were prepared in Mueller-Hinton broth for all the bacteria and incubated for 24 h at 37 ± 1°C. Testing was carried out in Mueller-Hinton broth at pH 7.4 and the serial dilution technique was applied. The microorganisms were grown overnight in Mueller-Hinton broth at 37 ± 1°C and the final inoculums size was 105 CFU ml^{-1} for the anti-bacterial assay. A set of tubes containing only inoculated broth was kept as controls. After incubation for 24 h at 37 ± 1°C, each experiment was duplicated to define the correct values. The results of anti-microbial potency for synthesized compounds are listed in **Table 2**.

Anti-fungal assay[17]

The anti-fungal activity of substituted- (tetrazolo[1,5-*a*] quinolin-4yl) derivatives **[BM1 to BM5]** was studied at 100ppm concentration assayed against the growth against *Aspergillus niger* and *Trichoderma ressi*at 100 ppm concentration in dimethyl sulfoxide (DMSO) and Griseofulvin was used as the standard **(Table 2)**.

The fungi were incubated for 24 h at 37 ± 1°C. The two-fold serial dilution technique was applied. The microorganisms were grown overnight in Triptic soy broth at 37 ± 1°C and the final inoculum size was 1 X 106 spore per mL for the antifungal assay. A set of tubes containing only inoculated broth was kept as a control.

After incubation for 48 h at 37 ± 1 °C, each experiment was duplicated in order to define correct values. Normal saline was used to make a suspension of spore of fungal strain for lawning. A loopful particular fungal strain was transferred to 3 mL saline to get a suspension of corresponding species. Twenty milliliter of agar media was poured into each Petri-dish. Excess of suspension was decanted and the plates were dried in an incubator for 48 h at 37 °C. Zones of Inhibition were measured in mm and compared with the Standard at 100 ppm concentration. The results for anti-fungal assay of compounds **[BM1 to BM2]** are described in **Table 2**.

VI.RESULT AND DISCUSSION

Table-1 : Synthesis of substituted benzimidazoles from aldehydes with 2-phenylenediamine.

Sl. No.	**Compound**	**Yield (%)**	**M. P.** (℃)
1	5-Methyl-2-(4-nitrophenyl)-1H-benzimidazole	95	101-103
2	5-Methyl-2-(3-methyphenyl)-1H-benzimidazole	77	73-75
3	5-Nitro-2-phenyl-1H-benzimidazole	81	167-169
4	5-nitro-2-(4-chlorophenyl)- 1H-benzimidazole	84	259-261
5	5-Nitro-2-(4-nitrophenyl)-1H-benzimidazole	86	87-89

Spectral Characterization of synthesized compound

5-methyl-2-(4-nitrophenyl)-1H-benzimidazole(BM1)

IR (KBr pallets): *V*max 3109, 1605, 1511, 1463, 1354, 1176, 739, 701 and 657 cm-1. **^{1}H NMR** (DMSO-*d6*): δ 8.39 (s, 4H+1H, overlapped Ar-H and N-H), 7.54 (d, J = 8.0 Hz, 1H, Ar-H), 7.43 (s, 1H, Ar-H), 7.09 (d, J = 8.3 Hz, 1H, Ar-H) and 2.44 (s, 3H, -CH3). **^{13}C NMR** (DMSO-*d6*): δ 159.0, 153.6, 143.2, 136.3, 131.0, 129.3, 127.9, 119.4, 114.7, 114.6, 111.5 and 31.1. **Mass** (EI, m/z): 254 [M+]

5-Methyl-2-(3-methylphenyl)-1H-benzimidazole (BM2)

IR (KBr): ν = 3323 (NH), 3059 (CHaromatic) 1448 (C=C), 1622 (C=N) cm-1.**^{1}H NMR** (CDCl3): δ 9.72 (s, 1H, N-H), 7.98 (s, 1H, Ar-H), 7.92 (s, 1H, Ar-H), 7.53 (d, J = 8.0 Hz, 1H, Ar-H), 7.41 (s, 1H, Ar-H), 7.28- 7.23(m, 1H, Ar-H), 7.18 (d, J = 7.6 Hz, 1H, Ar-H), 7.07 (d, J = 8.4 Hz, 1H, Ar-H), 2.45 (s, 3H, -CH3) and 2.21 (s, 3H, -CH3). **^{13}C NMR** (CDCl3): δ 151.9, 138.8, 138.7, 137.6, 132.8, 130.8, 129.7, 128.9, 127.5, 124.4, 123.8, 115.1, 114.5, 21.7 and 21.2. **Mass** (EI, m/z): 223.12 [M+]

5-Nitro-2-phenyl-1H-benzimidazole(BM3)

IR (KBr): ν= 3348 (NH), 3047 (CHaromatic), 1462 (C=C) cm-1. 1 HNMR (300 MHz, DMSO): δ ppm = 7.20-7.65 (m 7H, CHaromatic), 8.16-8.20 (m, 2H, CHaromatic), 12.93 (bs, 1H, NH)

5-nitro-2-(4-chlorophenyl)- 1H-benzimidazole(BM4)

IR (KBr): ν = 3377 (NH), 3061 (CHaromatic) 1440 (C=C), 1599 (C=N) cm-1. 1 HNMR (300 MHz, DMSO): δ ppm = 7.21 (q, J= 2.6 Hz, 2H, CHaromatic), 7.501-7.54 (m, 3H, CHaromatic), 7.66 (q, J= 3.8 2H, Hz, CHaromatic) 7.90 (t, 1 H, J= 9.4 Hz, CHaromatic), 12.73 (bs, 1H, NH).

5-Nitro-2-(4-nitrophenyl)-1H-benzimidazole(BM5)

IR (KBr): ν = 3367 (NH), 3061 (CHaromatic) 1516 (C=C), 1597 (C=N) cm-1. 1 HNMR (300 MHz, DMSO): δ ppm = 7.254-7.75 (m, 4H, CHaromatic), 8.39 (bs, 4H, CHaromatic), 13.30 (bs, 1 H, NH)

Table 2: Anti-microbial activity of substituted-(tetrazolo[1,5-*a*] quinoline-4yl) **[BM1-BM5]**

Compound	Zone of inhibition in mm					
	Anti-bacterial activity				Anti-fungal activity	
	Bacillus substilis	*Staphylo. aureus*	*Pseudomonas aeruginosa*	*Proteus vulgaris*	*Aspergillus niger*	*Tricho. Ressi*
BM1	14	14	14	11	16	15
BM2	14	12	12	10	8	8
BM3	12	13	14	11	10	11
BM4	11	12	12	8	8	8
BM5	9	11	10	8	7	8
Streptomycin	24	22	24	26	-	-
Griseofulvin	-	-	-	-	17	15

IV.CONCLUSION

The present study was aimed at synthesis and characterization of some novel substituted- (tetrazolo[1,5-*a*] quinolin-4yl) derivatives **[compound-1 to compound-5].** Different derivatives were synthesized by by condensation of different 2-phenylenediamines and substituted aldehyde in presence of Zirconyl nitrate as a heterogeneous Lewis acid catalyst in ethanol.. Most of the compounds are showed moderately activity when compared to standard drug. Compound **BM1** showed moderate activity against *S. aureus* and exhibited more activity against all bacteria. Compound **(BM5)** showed as inactive against gram positive bacteria. Compounds **(BM1)** and **(BM3)** displayed better antifungal activity against 100 µg/ml concentration,

REFERENCES

1. Gurer-Orhan H., Orhan H., Suzen S., Puskullu M. O., Buyukbingol E. *J. Enzyme Inhib. Med. Chem.* **2006**, *21*, 241.
2. (a) Ozkay Y., Tunai Y., Karaca H., Isikdag I. *Eur. J. Med. Chem.* **2010**, *45*, 3293. (b) Arjmand F., Mohani B., Ahmad S. *Eur. J. Med. Chem.* **2005**, *40*, 1103
3. (a) Sreena K., Ratheesh R., Rachana M., Poornima M., Shyni C. *Hygeia*, **2009**, *1*, 21. (c) Mavrova A. Ts., Anichina K. K., Vuchev D. I., Tsenov J. A., Denkova P. S., Kondeva M. S., Micheva M. K. *Eur. J. Med. Chem*. **2006**, *41*, 1412.
4. (a) Zienab M., NofalElsyed A., Soliman S. S., Abd El-Karim, Magdy I., Elzahar Aladdin M., Srour S. S., Timothy J. M., *Acta PoloniaePharmaceutica- Drug Research*, **2011**, *68*, 519. (b) Horton D. A., Bourne G. T., Smythe M. L. *Chem. Rev.* **2003**, *103*,

893. (c) Mann J., Baron A., Opoku-Boahen Y., Johansson E., Parkinson G., Kelland L. R., Neidle S. *J. Med. Chem.* **2001**, *44*, 138. (d) White A. W., Almassy R., Calvert A. H., Curtin N. J., Griffin R. J., Hostomsky Z., Maegley K., Newell D. R., Srinivasan S., Golding B. T. *J. Med. Chem.* **2000**, *43*, 4084.

5. (a) Mizuno C. S., Chittiboyina A. G., Shah F. H., Patny A., Kurtz T. W., Pershadsingh H. A., Speth R. C., Karamyan V. T., Carvalho P. B., Avery M. A. *J. Med. Chem.* **2010**, *53*, 1076. (b) Ogino Y., Ohtake N., Nagae Y., Matsuda K., Moriya M., Suga T., Ishikawa M., Kanesaka M., Mitobe Y., Ito J., Kanno T., Ishiara A., Iwaasa H., Ohe T., Kanatani A., Fukami T. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5010. (c) Kumar J. R., Jawaharand J., Pathak D. P. *E-Journal of Chemistry*, **2006**, *3*, 278.
6. (a) Mader, M., de Dios, A., Shih, C., Anderson, B. D. *Bioorg. Med. Chem. Lett*. **2008**, *18*, 179. (b) Lazer E. S., Matteo M. R., Possanza G. J. *J. Med. Chem.* **1987**, *30*, 726.
7. (a) Achar K. C., Hosamani K. M., Seetharamareddy H. R. *Eur. J. Med. Chem.* **2010**, *45*, 2048. (b) Elmer G. I., Pieper J. O., Goldberg S. R., George F. R. *Psychopharmacology (Berl.)*, **1995**, *117*, 23.
8. Katiyar S. K., Gordon V. R., Mclaughlin G. L., Edlind T. D. *Antimicrobial Agents and Chemotherapy*, **1994**, *38*, 2086.
9. Li Y. F., Wang G. F., He P. L., Huang W. G., Zhu F. H., Gao H. Y., Tang W., Luo Y., Feng C. L., Shi L. P., Ren Y. D., Lu W., Zuo J. P. *J. Med. Chem.* **2006**, *49*, 4790.
10. (a) Singh N., Pandurangan A., Rana K., Anand P., Ahamad A., Tiwari A. K. *International Current Pharmaceutical Journal*, **2012**, *1(5)*, 119. (b) Walia R., Hedaitullah M., Naaz S. F., Iqbal K., Lamba, H. *IJRPC*, **2011,** *1(3)*, 2231. (c) PK D., Naidu A., Reddy P., Kumar N., Vineel B. G. *Ind. J. Chem.* **2008**, *47*, 1443. (d) Cho S. Y., Kang S. K., Kim S. S., Cheon H. G., Choi J. K., Yum E. K. *Bull. Korean Chem. Soc.* **2001**, *22*, 1217. (e) Kim J. S., Gatto B., Yu C., Liu A., Liu F. L., Voie E. J. *J. Med. Chem.* **1996**, *39*, 992. (f) Yamada M., Yura T., Morimoto M., Harada T., Yamada K., Honma Y., Kinoshita M., Sugiura M. *J. Med. Chem.* **1996**, *39*, 596.
11. (a) Tonelli M., Paglietti G., Boido V., Sparatore F., Marongiu F., Marongiu E., LaColla P., Loddo R. *Chem. Biodivers.* **2008**, *5*, 2386. (b) Beaulieu P. L., Bousquet Y., Gauthier J., Gillard J., Marquis M., McKercher G., Pellerin C., Valois S., Kukolj G. *J. Med. Chem.* **2004**, *47*, 6884.
12. Sanja O., Podunavac-Kuzmanovi, Dijana J. B., Dragoljub D. *APTEFF*, **2007**, *38*, 139.
13. Shingalapur R. V., Hosamani K. M., Keri R. S., Hugar M. H. *Eur. J. Med. Chem*. **2010**, *4*, 1753.
14. Gualtieri F., Brody G., Fieldsteel A. H., Skinner W. A. *J. Med. Chem.* **1972**, *15*, 420.
15. (a) Rasid M., Husain A., Mishra R. *Eur. J. Med. Chem.* **2012**, *54*, 855. (b) Tamura Y., Omori N., Kouyama N., Nishiura Y., Hayashi K., Watanabe K., Tanaka Y., Chiba T., Yukioka H., Sato H., Okuno T. *Bioorg. Med. Chem.* **2012**, *22*, 5498.
16. Bauer, A. W.; Kirby, W. M.; Sherris, J. C. Am. J. Clin. Pathol. 1966, 39, 493-496.
17. Varma, R. S. Editor, Antifungal Agents: Past, Present and Future prospects, National Academy of Chemistry and Biology**,** Lucknow, India, 1998.